October 15, 2002

EXTRA! EXTRA! READ ALL ABOUT IT!

TOP STORIES:

**** There are FIVE new papers entering in-house review on this update (and one entering at the submitted stage)! Also, the special XHAB abstract section has been held-over for one more issue (in case you want to look at them before the meeting).

**** "NEW!!!" denotes papers that are new to the manuscript update or have switched categories (ex. Submitted to In Press)

** Remember! Entire manuscripts can be found in the biotoxins folder in "users on Spottail" (in the same folder as workshop presentations).

IN HOUSE REVIEW:

**** NEW!!! TYPE B BREVETOXINS SHOW TISSUE SELECTIVITY FOR VOLTAGE-GATED SODIUM CHANNELS: COMPARISON OF BRAIN, SKELETAL MUSCLE AND CARDIAC SODIUM CHANNELS.**

… Marie-Yasmine Bottein Dechraoui and John S. Ramsdell

ABSTRACT

Brevetoxins and ciguatoxins are two classes of phycotoxins which exert their toxic effect by binding to site-5 of voltage-gated sodium channels. Sodium channels, a family of at least ten structurally different proteins, are responsible for the rising phase of the action potential in membranes of neural, cardiac and muscular excitable cells. This work is a comparative study of the binding properties and the cytotoxic effect of ciguatoxins and brevetoxins on human embryonic cells (HEK) stably expressing either the skeletal muscle ($Na_v1.4$), or the cardiac ($Na_v1.5$) sodium channel α-subunit isoforms. We report that type A (PbTx-1) and type B (PbTx-3 and PbTx-2) brevetoxins as well as ciguatoxins target both cardiac and muscle channels; type B brevetoxins show isoform selectivity, presenting a lower affinity for the heart than the skeletal muscle channel. The lower selectivity of type-B brevetoxins for heart sodium channels may result from a more rigid backbone structure than is found in type-2 brevetoxins and ciguatoxins.

** NEW!!! **MEASUREMENT OF BREVETOXIN LEVELS BY RADIOIMMUNOASSAY OF BLOOD COLLECTION CARDS AFTER ACUTE, LONG-TERM AND LOW DOSE EXPOSURE IN MICE.**

… Ricky Woofter, M-Yasmine Bottein Dechraoui, Ian Garthwaite,
Neil R. Towers, Christopher J. Gordon , José Córdova and John S. Ramsdell

ABSTRACT

A radioimmunoassay (RIA) has been developed using a sheep anti-brevetoxin to evaluate detection of brevetoxin on blood collection cards from mice treated with the brevetoxin congener (PbTx-3). The RIA was designed in similar format to receptor assay to facilitate comparison with previous work with blood collection cards. The RIA uses a 1/4000 dilution of sheep antiserum, 0.4 nM [^{3}H]-PbTx-3, and goat antisheep IgG-cellulose with separation on glass fiber filters. The receptor binding assay (RBA), using rat brain membrane, has an affinity for PbTx-3 (EC_{50}= 4.3 ± 1.5 nM, n=7) and recognizes type 1 and type 2 brevetoxins, as well as ciguatoxin. Whereas the RIA, using a PbTx-2 specific antibody, has an affinity for PbTx-3 (EC_{50}= 1.2 ± 0.2 nM, n=10) and recognizes both type 1 and type 2 brevetoxins, but not ciguatoxin. Comparison of the different brevetoxin subtypes affinity using RIA and RBA yields a rank order of potency where PbTx 6 > 3 = 2 = 9 > 1. Thus, the two assays provide comparable values for the commonly occurring PbTx-2 and 3 as well as PbTx-9, while showing differences for PbTx-6 and PbTx-1. We next compared the two assays by measuring brevetoxin in the blood of mice exposed to a sublethal dose, 180 µg/kg of PbTx-3 for 0.5, 1, 2, 4, and 24 hr. The blood from each mouse was preserved on blood collection cards. Each 0.1 ml blood spot was extracted in 2 ml methanol. This extract was then tested by both assays. The RBA reported the blood brevetoxin activity (at 2 hr brevetoxin activity was detected in 3 of 4 mice), while the RIA gave blood brevetoxin levels (at 2 hr: 25.75, 28.27, 39.26, 28.51 nM PbTx-3). Taken together these results show the value of tier-based testing for brevetoxin: antibody methods provide a good screening method that may detect metabolites; receptor-based methods provide a good toxicity measurement and LC-MS/MS provides absolute confirmation of toxin congeners.

** NEW!!! RE-EVALUATION OF PARALYTIC SHELLFISH TOXIN PRODUCTION BY BACTERIA ASSOCIATED WITH DINOFLAGELLATES OF THE PORTUGUESE COAST.

… Claudia A. Martins et al. (Greg Doucette)

ABSTRACT

Paralytic Shellfish Toxins (PSTs) are a suite of potent neurotoxins whose production is associated with certain dinoflagellate and cyanobacterial species. The autonomous production of PSTs by some bacterial strains, namely those associated with PST producing dinoflagellates, remains controversial. In addition to reports on their PST production, there is some evidence to suggest that certain compounds in some bacterial isolates were incorrectly identified as PSTs by HPLC analysis. In the current study, PST production by two bacterial strains, *Pseudomonas stutzeri* and *Pseudomonas diminuta*, isolated from *Alexandrium lusitanicum* and *Gymnodinium catenatum*, respectively, was evaluated using a mouse neuroblastoma (MNB) assay and the results compared to HPLC analyses of the same samples. Since we have previously assessed the presence of PSTs in these bacterial isolates by HPLC, results of the present study are also discussed in relation to our earlier findings.

Toxicity studies were performed under optimal conditions for toxin production and detection, as described in the published literature. PSTs were not detected by HPLC analysis in either supernatants or bacterial cell extracts. Analysis by MNB assay was negative for supernatants but initially positive for crude extracts. Nonetheless, this positive assay response was eliminated following C18 sep-pak clean-up of the extracts, indicative of a matrix effect on the assay and thus the absence of PSTs in these samples. We conclude that neither our MNB nor HPLC data are consistent with autonomous bacterial PST production under the study conditions.

** NEW!!! GTX_4 IMPOSTERS: CHARACTERIZATION OF FLUORESCENT COMPOUNDS SYNTHESIZED BY *Pseudomonas stutzeri* SF/PS AND *Pseudomonas/Alteromonas* PTB-1, SYMBIONTS OF SAXITOXIN-PRODUCING *Alexandrium* SPP.

… Tracie R. Baker et al. (Greg Doucette and Christine Powell)

ABSTRACT

Saxitoxins, the etiological agent of paralytic shellfish poisoning, are synthesized by dinoflagellates and cyanobacteria. Several reports indicate that bacteria are capable of saxitoxin synthesis. Two bacterial strains were isolated from saxitoxin-producing dinoflagellates, *Alexandrium tamarense* and *A. lusitanicum* (=*A. minutum*), and grown under a variety of culture conditions including those previously reported to induce saxitoxin synthesis in bacteria. Five fluorescent compounds were accumulated by the bacteria that had HPLC-FLD retention times similar to a reference standard of GTX_4, one of the saxitoxin congeners. However, we were unable to detect GTX_1, the epimeric partner of GTX_4, in the bacterial samples. The GTX_4 standard was hydrolyzed by NaOH/heat treatment but four of the bacterial compounds were stable. Unlike GTX_4, none of the five bacterial compounds were detectable by HPLC-FLD following electrochemical oxidation. The fluorescence emission spectrum of each of the five bacterial compounds was unique and readily discernable from the spectrum of GTX_4. None of the samples containing the putative GTX_4 toxin yielded positive results when analyzed by a ^{3}H-saxitoxin receptor-binding assay for saxitoxin-like activity. We cannot rule out the possibility that these bacteria produce saxitoxins; however, our data clearly demonstrate that they accumulate at least five different fluorescent compounds that could be easily mistaken for GTX_4. We conclude that these five fluorescent compounds are GTX_4 imposters and that fluorescence scanning and chemical/heat stability should, at a minimum, be incorporated into HPLC-FLD protocols for identification of saxitoxins.

** NEW!!! LEARNING IMPAIRMENT CAUSED BY A TOXIN PRODUCED BY *Pfiesteria piscicida* INFUSED INTO THE HIPPOCAMPUS OF RATS

… Edward D. Levin et al. (Peter D. R. Moeller and John S. Ramsdell)

ABSTRACT

Pfiesteria piscicida, an estuarine dinoflagellate, which has been shown to kill fish, has also been associated with neurocognitive deficits in humans. With a rat model, we have demonstrated the cause-and-effect relationship between *Pfiesteria* exposure and learning impairment. In several studies, we have replicated the finding in Sprague-Dawley rats that exposure to fixed acute doses of *Pfiesteria* cells or filtrates caused radial-arm maze learning impairment. Recently, this finding of *Pfiesteria*-induced learning impairment in rats has been independently replicated in another laboratory as well. We have demonstrated significant *Pfiesteria*-induced learning impairment in both the win-shift and repeated acquisition tasks in the radial-arm maze and in reversal learning in a visual operant signal detection task. These learning impairments have been seen as long as 10 weeks after a single acute exposure to *Pfiesteria*. In the current study, we used a hydrophilic toxin isolated from clonal *Pfiesteria piscicida* cultures (PfTx) and tested its effect when applied locally to the ventral hippocampus on repeated acquisition of rats in the radial-arm maze. Toxin exposure impaired choice accuracy in the radial-arm maze repeated acquisition procedure. The PfTx-induced impairment was seen at the beginning of the session and the early learning deficit was persistent across six weeks of testing after a single administration of the toxin. Eventually with enough practice each session the PfTx exposed rats did learn that session's problem as did control rats. This model has demonstrated the cause-and-effect relationship between exposure to a hydophillic toxin produced by *P. piscicida* and learning impairment and specifically that the ventral hippocampus was critically involved.

**** α,β,γ TUBULIN, THE MINIMAL SET OF TUBULIN REQUIRED TO DEFINE MICROTUBULE FUNCTION IN EUKARYOTIC CELLS ARE PRESENT IN THE UNICELLULAR DINOFLAGELLATE *Karenia brevis*.** … Michèle Barbier et al. (Jeanine Miller, Steve Morton and Fran VanDolah)

ABSTRACT

Tubulin is a highly conserved family of proteins that are a major component of the microtubule cytoskeleton of eukaryotic cells. Here we report the presence of the three essential members of this family, α-, β- and γ-tubulin, in the unicellular dinoflagellate *Karenia brevis* by western blotting and immunolocalization. The cortical cytoskeleton and the intracytoplasmic structures are detailed by immunocytofluorescence techniques using antibodies to each tubulin on whole-permeabilized cells from laboratory cultures or field samples. The cortical microtubules could be visualized with anti-α- and anti-β-tubulin labeling revealing a morphology typical of dinoflagellates, while γ-tubulin was detected near the nucleus, probably associated with the archoplasmic sphere. The mitotic spindle, which arises from this region is described during different stages of mitosis. The cortical cytoskeleton does not depolymerize during mitosis, a feature that appears to be unique to dinoflagellates. For the first time, a detailed description of the cytoskeleton and the mitotic process is presented in the dinoflagellate *K. brevis*.

**** POSSIBILITY OF DIARRHETIC SHELLFISH POISONING ASSOCIATED WITH *Prorocentrum lima (Dinophyceae)* IN PATAGONIAN GULFS (ARGENTINA).** … Ana María Gayoso et al. (Stacie Dover, Steve Morton, Mark Busman and Peter Moeller)

ABSTRACT

A massive DSP intoxication caused by the consumption of mussels harvested in the gulfs San José and Nuevo, Patagonia, Argentina occurred in March (autumn) 1999. The dinoflagellate Prorocentrum lima (Ehrenberg) Dodge was detected in net samples and in the stomach contents of the ribed mussel Aulacomya atra (Molina) and Mitylus edulis platensis (d'Orbigny). Extracts for both mussel species were positive for DSP-like activity using the fluorometric phosphatase inhibition assay. When the Mytilus and Aulacomya extracts were analyzed using the liquid chromatography and tandem mass spectrometry only dinophysis toxin-1 (DTX-1) was measured in the hydrolized samples while no peaks of okadaic acid (OA) or dinophysis toxin-1 were observed in the non-hydrolized samples. Aulacomya atra contained 94 ng DTX-1 per gram of the whole tissue and Mytilus edulis platensis, 21.2 ng DTX-1 per gram of the whole tissue. This was the first observation of DSP toxins in the Argentine coast.

**** A RECEPTOR BINDING ASSAY FOR PARALYTIC SHELLFISH POISONING TOXINS: OPTIMIZATION AND INTERLABORATORY COMPARISON** … Ruberu et al. (Greg Doucette and Christine Powell)

ABSTRACT

A receptor binding assay (RBA) for detection of paralytic shellfish poisoning toxins was formatted for use in a high throughput detection system employing microplate scintillation counting. The RBA technology was transferred from the National Ocean Service (NOS), which uses a Wallac TriLux 1450 MicroBeta microplate scintillation counter, to the California Department of Health Services (CDHS), which uses a Packard TopCount instrument. Due to differences in the detector arrangement between these two counters, markedly different counting efficiencies were exhibited, requiring optimization of the RBA protocol for the TopCount instrument. Precision, accuracy, and sensitivity (LOD = 0.2 mg STX equiv./100 g shellfish tissue) of the modified protocol were equivalent to those of the original protocol. The RBA robustness and adaptability were demonstrated by an interlaboratory study, in which STX concentrations in shellfish generated by the TopCount were consistent with MicroBeta-derived values. Comparison of saxitoxin reference standards obtained from the FDA and the National Research Council, Canada showed no observable differences. This study confirms the RBA's value as a rapid, high throughput screen prior to testing by the conventional mouse bioassay (MBA) and suitable for providing an early warning of increasing PSP toxicity when toxin levels are below the MBA limit of detection.

**** PHYSIOLOGICALLY DIVERSE CLONES OF *Karenia brevis* (DINOPHYCEAE) FROM THE GULF OF MEXICO HAVE IDENTICAL RIBOSOMAL DNA SEQUENCES.** … Loret et al. (Steve Morton and Mark Busman)

ABSTRACT

Maximum growth rate and toxin content were significantly different among five strains of *Karenia brevis* isolated from Texas and Florida when grown under the same culture conditions. Sequence analysis of the 18S rRNA gene and internal transcribed spacer (ITS) regions, however, revealed all strains were identical. A clear genetic basis for physiological variability among various geographical isolates from the Gulf of Mexico could not be assessed using these simple genetic markers; however, this study supports an emerging pattern of highly conserved 18S rDNA and ITS regions in species of phytoplankton that form toxic blooms.

**** QUALITATIVE AND QUANTITATIVE ANALYSIS OF CERAMIDE AND DIHYDROCERAMIDE SPECIES USING NORMAL PHASE HPLC COUPLED TO APCI-MS** … Pettus et al. (Mark Busman and Peter Moeller)

ABSTRACT

Not available at this time (see Mark Busman)

SUBMITTED:

** NEW!!! INVITED REVIEW!!! TOXIC *PFIESTERIA*: REVIEW OF THE SCIENCE AND COMMENT ON BERRY ET AL. (Proceedings of the Natural Academy of Sciences)

… J. M. Burkholder et al. (J. S. Ramsdell, P. D. R. Moeller and S. L. Morton)

ABSTRACT

Numerous studies have demonstrated the ability of *Pfiesteria* spp. to kill fish by physical attack, and by toxin without physical contact. Here we summarize research from more than 400 appropriately cultured toxic *Pfiesteria* strains, including data for fish death and impacts on mammals from *Pfiesteria* toxin. There is strong precedent for a range in toxicity among strains within a given toxic algal species; similarly, *Pfiesteria* strains range from non-inducible (incapable of killing fish with toxin) to highly toxic. These reports and data show that the conclusion presented in a recent paper in *PNAS* by Berry et al., that *Pfiesteria* species are not toxicogenic based on one strain, is in error because the culture protocols used by Berry et al. would not have allowed a toxic strain to express toxicity.

**** NON-CYCLIC EXPRESSION OF A CYCLIN B HOMOLOGUE IN THE PRIMITIVE DINOFLAGELLATE, *K. brevis*** … Michèle Barbier et al. (Tod Leighfield and Fran VanDolah)

ABSTRACT

The eukaryotic cell cycle is driven by a set of cyclin dependent kinases associated with their regulatory partners the cyclins, which confer activity, substrate specificity and proper localization of the kinase activity. We describe the cell cycle of *Karenia brevis* and provide evidence for the presence of a cyclin B homologue in this primitive eukaryotic dinoflagellate. This cyclin B homologue has an unusual behavior, since its expression is permanent and its localization is cytoplasmic throughout the cell cycle. This behavior is similar to a cyclin B homologue, p56, previously described in a different species of dinoflagellate. However, in *K. brevis*, the cyclin B homologue is also

present in the nucleus, specifically bound to the nucleolus during interphase. There is no evidence for the translocation to the nucleus during mitosis. Here we discuss the unique behavior of the cyclin B homologue in dinoflagellates, its relationship to the unusual characteristics of dinomitosis, and its potential implications regarding the evolution of cell cycle regulation among eukaryotes.

**** MICROBIAL COMMUNITY INTERACTIONS AND POPULATION DYNAMICS OF AN ALGICIDAL BACTERIUM ACTIVE AGAINST *Karenia brevis* (Dinophyceae)** … Xavier Mayali and Greg Doucette (Harmful Algae)

ABSTRACT

We investigated the population dynamics of *Cytophaga* strain 41-DBG2, a bacterium algicidal to the harmful algal bloom (HAB) dinoflagellate *Karenia brevis*, in laboratory experiments using fluorescent *in situ* hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE). Following its introduction into non-axenic *K. brevis* cultures at concentrations of 10^3 or 10^5 cells ml^{-1}, 41-DBG2 increased to 10^6 cells ml^{-1} before the initiation of its algicidal activity. Such threshold concentrations were not achieved when algal cell numbers were low, suggesting that the growth of this bacterium required high levels of dissolved organic matter (DOM) excreted by the algae. It remains to be determined whether the threshold concentration is required to trigger an algicidal response by 41-DBG2 or alternatively, is the point at which the bacterium accumulates to an effective killing concentration. The microbial community, as determined by DGGE profiles, did not change until after *K. brevis* cells were in the process of lysing, indicating a response to the rapid input of algal-derived organic matter. We found that the resistance to algicidal attack exhibited by several *K. brevis* clones was due to the inhibition of 41-DBG2 growth in the presence of currently uncultivable bacteria associated with those clones, consequently preventing 41-DBG2 from reaching its threshold concentration required for algicidal activity. Remarkably, immunity and susceptibility to the algicidal activity of 41-DBG2 could be exchanged between *K. brevis* clones with the transfer of their respective unattached bacterial communities, and several dominant phylotypes sequenced from these communities belonged to the α-Proteobacteria, γ-Proteobacteria, and Cytophaga-Flavobacterium-Bacteroides (CFB) groups. We hypothesize that the CFB bacteria may be successfully competing with 41-DBG2 (also a member of the CFB) for nutrients, thereby inhibiting the growth of the latter and indirectly providing immunity against algicidal attack. We conclude that if algicidal bacteria play a significant role in HAB dynamics, as some authors have inferred, bacterial community interactions are crucial factors that must be taken into consideration in future studies.

**** *Emerita analoga* (STIMPSON)- POSSIBLE NEW INDICATOR SPECIES FOR THE PHYCOTOXIN DOMOIC ACID IN CALIFORNIA COASTAL WATERS** …M. E. Ferdin et al. (Christine Powell and Greg Doucette) (Toxicon)

ABSTRACT

Blooms of domoic acid (DA) synthesizing diatoms (*Pseudo-nitzschia* spp.) have been associated with the death and injury of hundreds of marine shorebirds and mammals, exposed humans to potentially serious health risks, and threatened to significantly impact coastal fisheries and economies dependent on marine resources. While indicator organisms are widely utilized to monitor for marine biotoxins like paralytic shellfish poisoning (PSP) toxins, a reliable intertidal indicator species to monitor DA remains to be identified. Here we evaluate and confirm the utility of the common sand crab (*Emerita analoga*) as an indicator for DA in comparison with sea mussels (*Mytilus californianus*). Mussels and sand crabs, collected from natural populations in Santa Cruz, California (Apr. 1999 - Feb. 2000), were tested for DA using the HPLC-UV method. Toxin loads in sand crabs ranged from below detectable limits to 10.4 µg DA g^{-1} and coincided with the abundance of DA producing *Pseudo-nitzschia* species nearshore. Toxin levels in mussels collected during the study period were below HPLC-UV detectable limits. The rise and fall of DA in sand crabs in synchrony with *Pseudo-nitzschia* abundance, combined with this common intertidal species' accessibility and ease of DA extraction, recommend sand crabs as a reliable, cost-effective monitoring tool for DA in the coastal environment.

**** PHYLOGENETICS AND rRNA PROBE DESIGN FOR ALGICIDAL BACTERIA ACTIVE AGAINST *Karenia brevis* (Dinophyceae)** … Xavier Mayali and Greg Doucette (Aquatic Microbial Ecology)

ABSTRACT

Two strains of bacteria isolated from the Gulf of Mexico and determined to be algicidal against the harmful algal bloom (HAB) forming dinoflagellate *Karenia brevis* were phylogenetically classified using 16S rDNA data. A novel statistical analysis of these strains as well as additional algicidal bacteria associated with blooms of other HAB species revealed 3 phylogenetically distinct clades abundant in such bacteria, within the genera *Cytophaga*, *Alteromonas*, and *Pseudoalteromonas*. This pattern is consistent with the hypothesis that independent radiation events took place during the course of algicidal bacteria evolution, suggesting that algicidal activity could have been an adaptive trait, and supporting the idea that algicidal bacteria may play a role in algal bloom dynamics. A strain-specific rRNA probe was designed for one algicidal bacterium (strain 41-DBG2) and using fluorescent *in situ* hybridization (FISH), was successful in identifying this strain in laboratory and field samples enriched for algicidal bacteria. In addition, one sample from a natural *K. brevis* bloom exhibited a positive signal using this method. Denaturing gradient gel electrophoresis (DGGE) was applied as an additional culture-independent method capable of identifying algicidal bacterium 41-DBG2 in mixed bacterial assemblages. DGGE also revealed more complex microbial communities in *K. brevis* bloom samples compared to 6 laboratory clonal isolates. Several of the latter also appeared to share phylotypes with one another, suggesting that similar bacteria may be associated with *K. brevis* cultures originating from different locations.

IN PRESS:

****** NEW!!! **EVIDENCE FOR A CAMP-DEPENDENT PROTEIN KINASE IN A DINOFLAGELLATE, *Amphidinium operculatum*** … Tod Leighfield et al. (Michèle Barbier and Fran M. Van Dolah)

ABSTRACT

A cAMP dependent protein kinase (PKA) was identified in the dinoflagellate *Amphidinium operculum*. *In vitro* kinase activity towards kemptide, a PKA-specific substrate, was not detectable in crude lysates. However, fractionation of dinoflagellate extracts by gel filtration chromatography showed PKA-like activity toward kemptide at approximately 66 kDa. These findings suggest that possible low molecular weight inhibitors in crude lysates were removed by the gel filtration chromatography. Pre-incubation of extracts with cAMP prior to chromatography resulted in an apparent molecular weight shift in the *in vitro* kinase assay to 40 kDa. An in-gel kinase assay reflected activity of the free catalytic subunit at approximately 40kDa. Furthermore, Western blotting with an antibody to the human PKA catalytic subunit confirmed a catalytic subunit with a mass of approximately 40kDa. Results from this study indicate that the PKA in *A. operculatum* has a catalytic subunit of similar size to that in higher eukaryotes, but with a holoenzyme of a size suggesting a dimeric, rather than tetrameric structure.

****** NEW!!! **SODIUM CHANNEL ISOFORM-SPECIFIC TOXINS, IMPLICATION FOR TOXICOLOGICAL ANALYSIS** … Marie-Yasmine Bottein Dechraoui and John S. Ramsdell

ABSTRACT

Arrays of biological compounds exert their toxic effect by binding to specific sites on voltage-gated sodium channels, providing potent chemical agents for defense or to kill their prey, but also inducing potent human intoxication or envenoming. Probably present in cells of all life forms, sodium channels are expressed to a greater extent in nerve, heart and skeletal muscle cells where they play a central role in the generation and propagation of action potentials. In mammals, a family of ten sodium channel α-subunits has been documented through isolation of separate cDNA and subsequent amino acid assignment. Sodium channels can be differentiated by their primary structure, their kinetics as well as their pharmacologic properties such as their relative sensitivity to a specific neurotoxin. They were initially distinguished as two subtypes according to their sensitivity to tetrodotoxin. However, through the expression of functional sodium channel subtypes in cell systems, other toxins also reveal further selectivity. This review of toxin interaction with voltage gated sodium channels, examines toxin binding sites, toxin selectivity for channel isoforms and implications of emerging research for toxiciological analysis

**** IMPACTS OF ALGAL TOXINS ON MARINE MAMMALS.**…Fran VanDolah et al. (Greg Doucette). (Book chapter in Toxicology of Marine Mammals)

ABSTRACT

Not available at this time (see Fran)

**** PARALYTIC SHELLFISH POISONING TOXINS IN THE ABALONE *Haliotis midae* ON THE WEST COAST OF SOUTH AFRICA**[5]…Grant C. Pitcher et al. (Greg Doucette and Christine Powell) (Journal of Shellfish Research)

ABSTRACT

In April 1999, monitoring on two abalone farms on the West Coast of South Africa provided evidence of the presence of PSP toxins in the cultured abalone *Haliotis midae*. Subsequent analysis of wild animals collected from the West Coast also revealed the accumulation of PSP toxins in these gastropods. The toxicity of individual animals as measured by the AOAC mouse bioassay showed considerable variation, ranging from below the assay detection limit to a maximum of 1609 µg STX eq 100 g^{-1}. Initial observations found PSP toxins in abalone to be coincident with blooms of *Alexandrium catenella* indicating that this dinoflagellate was the probable cause of abalone toxicity. Subsequent detection by receptor binding assay, of toxicity in abalone on the South Coast, an area considered free of *A. catenella* blooms, casts some doubt as to the source of the toxins. The toxin composition in the abalone as determined by HPLC was dominated by STX, and differed significantly from the toxin profile of *A. catenella* and the co-occurring mussel, *Mytilus galloprovincialis*. These findings indicated either a high capacity for biotransformation of PSP toxins by abalone or that *A. catenella* was not the source of the toxin. Investigation of the anatomical distribution of toxins revealed that they were not evenly distributed throughout the abalone tissues, but appeared to concentrate in outer layer tissue. The muscular foot made a disproportionately low contribution to the total toxin content of the mollusc, whereas the epipodial fringe, although comprising a small proportion of the abalone total weight, contributed substantially to the total toxin content. The epipodial fringe is typically included with the muscular foot as that part of the animal marketed for human consumption. The negative impacts of PSP contamination on abalone spawning and larval survival are presented and the findings of this study are compared to observations of PSP toxins in the abalone *Haliotis tuberculata* on the Galician coast. The inability of abalone to detoxify or depurate accumulated PSP toxins below the regulatory level threatens the future of the established abalone fishery and the newly developed aquaculture operations on the West Coast of South Africa.

**** CULTURE METHODS (**Chapter in Manual of Harmful Marine Microalgae**)**…R. R. L. Guillard and S.L. Morton

ABSTRACT

Not available in electronic form at this time. See Steve Morton.

**** KRILL: A POTENTIAL VECTOR FOR DOMOIC ACID IN MARINE FOOD WEBS**… Sibel Bargu et al. (Christine Powell and Greg Doucette) (Marine Ecology Progress Series)

ABSTRACT

Over the past decade, blooms of the domoic acid (DA)-producing diatom *Pseudo-nitzschia* have been responsible for numerous deaths of marine mammals and birds in Monterey Bay, CA. Pacific euphausiids (krill) are important members of the local zooplankton grazer community and comprise the primary diet of squid, baleen whales, and many seabirds. Krill are thus a key potential vector for the transfer of DA to higher trophic level organisms in Monterey Bay. A better understanding of the quantitative trophic interactions and body burden of DA in krill is required to predict whether they can act as an effective vector for this neurotoxin. Here we report results of toxin analyses and gut content examinations of krill, collected from Monterey Bay, CA, in 2000. Corresponding counts of toxic *Pseudo-nitzschia* species in the water and their cellular DA concentrations were also obtained at the collection sites. Toxin analysis by receptor binding assay demonstrated that DA in krill tissue varied between 0.1 – 44 µg DA equiv g^{-1} tissue (confirmed by tandem mass spectrometry) depending upon the abundance of toxic *Pseudo-nitzschia* species in the water. The occurrence of *Pseudo-nitzschia* frustules in the digestive tract of krill verified that a toxic

species of this diatom was an important part of their diet and thus implicated this phytoplankter as the source of domoic acid. These findings provide, for the first time, compelling evidence for the role of krill as a potential transfer agent of the phycotoxin DA to higher trophic levels in marine food webs.

**** USE OF CELL-SPECIFIC PAM-FLUOROMETRY TO CHARACTERIZE HOST SHADING IN THE EPIPHYTIC DINOFLAGELLATE *Gambierdiscus toxicus*** …Tracy A. Villareal and Steve L. Morton (Journal of Marine Ecology)

ABSTRACT

Cell-specific fluorescence characteristics were used to characterize the light tolerance of the toxic benthic dinoflagellate *Gambierdiscus toxicus*. The fluorescence parameter F_v:F_m was measured on individual cells collected from foliose red algae growing in the sub-tidal margin of Southwater Cay, Belize. Samples were collected over several days during sunny and cloudy conditions and compared to samples *in-situ*. The data from individual cells was used to generate both frequency histograms and averages. Maximum individual cell (F_v:F_m) values reached 0.81 in pre-dawn samples, a value near the theoretical maximum for PAM fluorometry. In field samples, average F_v:F_m declined only slightly during the day, but cells incubated under 47% sunlight showed a significant mid-day depression. In freshly collected samples, near-maximum F_v:F_m could be found in individual cells at all time points; however, the frequency histograms indicated a great range in F_v:F_m at all time points. In contrast, cultures showed a tight distribution around a mean. Field samples showed a rapid recovery to near maximum F_v:F_m when assayed using a standardized actinic light series. Similar results were found in laboratory culture grown at 73 µmol m^{-2} s^{-1}, but not at 383 µmol m^{-2} s^{-1}. These data provide empirical support for suggestions that *G. toxicus* exploits the 3-dimensional structure of the algal host thallus to minimize light. This strategy permits *G. toxicus*, a high-light intolerant species, to thrive in shallow, well-lit tropical seas.

****DEVELOPMENT OF A PROTOCOL FOR DETERMINATION OF DOMOIC ACID IN MOLE CRABS (*Emerita analoga*): A POSSIBLE NEW INDICATOR SPECIES**[4]…Christine Powell et al. (Toxicon 40:481-488)

ABSTRACT

The aim of this study was to begin evaluating the utility of mole crabs (*Emerita analoga*) as an indicator species for the algal neurotoxin, domoic acid (DA), in Monterey Bay, California, USA, a site of recurrent blooms of the DA-producing diatom *Pseudo-nitzschia*. One of the current sentinel organisms, the intertidal blue mussel (*Mytilus edulis*), shows minimal or undetectable toxicity during some local bloom events. As a critical step in assuring the accuracy of DA determinations in *E. analoga*, we have developed and validated a highly efficient extraction protocol that yields toxin recoveries of 97 $\pm$ 2.9 percent. We also determined by HPLC-UV and receptor binding assay, with confirmation by LC-MS/MS analyses, that mole crabs accumulated measurable amounts of DA during toxic *Pseudo-nitzschia* blooms, while the blue mussel showed no detectable toxin. In addition, a comparison of inter-animal variability (n = 60) in DA content revealed values ranging over an order of magnitude (*ca*. 0.5 to 5 micrograms DA/g tissue) and no consistent trend with size class, based on either animal weight or length. These data on the toxicity of individual animals will be useful in designing an appropriate sampling strategy for monitoring DA and, importantly, indicate that mole crabs do not appear to progressively bioaccumulate DA with age.

**** CHARACTERIZATION OF THE IONIC PROPERTIES OF A PURINERGIC RECEPTOR ($P2X_7$) IN GH_4C_1 RAT PITUITARY CELLS: EFFECTS OF A BIOACTIVE SUBSTANCE PRODUCED BY *Pfiesteria piscicida*…** Ana Clara Melo et al.

ABSTRACT

Pfiesteria picicida is a toxic dinoflagellate that leads to fish and human toxicity. It produces a bioactive substance that leads to cytotoxicity of GH_4C_1 rat pituitary cells. Extracellular ATP acting on P2X7 purinergic receptors induces the formation of a non-selective cation channel**,** causing elevation of the cytosolic free calcium followed by a characteristic permeabilization of the cell to progressively larger ions and subsequent cell lysis. Here we investigated whether GH_4C_1 rat pituitary cells express functional P2X7 receptors**,** and if so, are they activated by a bioactive substance isolated from toxic *Pfiesteria piscicida* cultures. We tested the selective agonist 2′-3′-(O)-(benzoyl-4-benzoyl)-ATP (BzATP) and antagonists piridoxalphosphate-6-azophenyl-2′-4′-disulphonic acid (PPADS) and oxidized-ATP (oxATP) using elevated cytosolic free calcium in Fura-2 loaded cells, and induced

permeability of these cells to the fluorescent dye YO-PRO-1 as endpoints. We demonstrated that in GH_4C_1 cells, BzATP induces both the elevation of cytosolic free calcium and the permeabilization of the cell membrane. ATP induced membrane permeabilization was inhibited by PPADS reversibly and by oxATP irreversibly. The putative *Pfiesteria* toxin (pPfTx) also elevated cytosolic free calcium in Fura-2 in GH_4C_1 cells**,** and increased the permeability to YO-PRO-1 in a manner that was inhibited fully by oxATP. This study indicates that GH_4C_1 cells express a purinoceptor with characteristics consistent with the P2X7 subtype**,** and that pPfTx mimics the kinetics of cell permeabilization by ATP.

XHAB 2002 ABSTRACTS:

**** DETECTION AND QUANTIFICATION OF MARINE TOXIN EXPOSURE USING BLOOD COLLECTION CARDS** … Marie-Yasmine Bottein Dechraoui et al. (Stacie Dover, Ricky Woofter, Peter Moeller and John Ramsdell)

ABSTRACT

Many accurate, rapid and readily performed methods have been developed for the detection of marine biotoxins, but their application for seafood poisoning monitoring and for exposed mammalian or human diagnostics remain a challenge. Indeed, detection implies a direct measurement of toxins in complex matrices such as fluids or tissue and the presence of multiple metabolites often with different pharmacological properties. We have developed a method to biomonitor brevetoxin and okadaic acid exposure using blood, collected, dried and stored on cellulose blood collection cards (0.1 ml of blood per spot). The toxin extraction procedure from the blood spot, estimated using spiked blood of brevetoxin (mice and fish blood) or okadaic acid (mice and turtle blood) gave linear and efficient recovery. Moreover our extract induced low matrix interference on the existing detection methods. Less than 20% quenching effect was measured with receptor binding and radioimmunoassay for blood brevetoxin detection and also less than 20% interference on protein phosphatase fluorimetric assay for blood okadaic acid detection. These highly sensitive detection methods allowed us to easily measured brevetoxins in mice 4 hrs after ip or oral sublethal exposure (180 ug/kg) and comparable studies will be conducted with okadaic acid. This sample collection method, widely employed for routine diagnostic and genetic testing of newborns provides a method for toxins identification in living animals as a means to assess exposure during unusual events associated with harmful algal blooms. We are evaluating this biomonitoring method for additional marine toxins and anticipate that this approach will provide a method for diagnostic of human intoxication.

**** REMOTE DETECTION OF HAB SPECIES USING THE ENVIRONMENTAL SAMPLE PROCESSOR (ESP): PROGRESS AND FUTURE DIRECTIONS** … Scholin et al. (Greg Doucette)

ABSTRACT

Molecular probes are extremely useful tools for identifying water borne microorganisms and the substances they produce, even when those targets are very dilute and embedded in a taxonomically complex and organically rich matrix. Application of such technology outside of a laboratory poses many technological challenges, particularly if unattended, real-time synoptic analysis of multiple locations for extended periods of time is desired. The Environmental Sample Processor (ESP) is a novel instrument developed in an effort to meet these demands. The ESP collects discrete water samples, concentrates microorganisms and automates application of DNA (or other) molecular probes to enable identification and quantification of particular species captured. The instrument transmits results of DNA probe array-based assays in near real-time to a shore-based location for processing, interpretation and dissemination. In addition, the ESP archives discrete samples for nucleic acid, microscopic and toxin analyses for verifying and augmenting near real-time data from the probe arrays as well as facilitating discovery-based analyses in the laboratory. This presentation will focus on field tests of the ESP system, including remote, subsurface detection of *Alexandrium tamarense* in the Gulf of Maine.

**** THE MITOTIC PROCESS AND REGULATION OF THE CELL CYCLE IN THE FLORIDA RED TIDE DINOFLAGELLATE *Karenia brevis*…** Michèle Barbier et al. (Jeanine Miller and Fran VanDolah)

ABSTRACT

Although dinoflagellates are eukaryotic organisms which emerged close to ciliates and apicomplexa, they have a unique form of mitosis termed dinomitosis, in which the nuclear membrane does not break down, but an extranuclear mitotic spindle forms that goes through the nucleus via cytoplasmic channels. Here we provide a detailed description of the morphology of the nucleus, chromatin and microtubular cytoskeleton of *K. brevis,* during interphase (G1, S) and mitotic stages (prophase, metaphase, anaphase, telophase) of the cell cycle, and we describe the cell cycle regulatory proteins that control progression through these stages. *K. brevis* expresses three forms of tubulin that are the minimum complement required to define microtubule function in eukaryotic cells: α, β and γ-tubulin. γ-tubulin is localized to the microtubule organizing centers, whereas α- and β-tubulin make up both the cytoskeleton and the mitotic spindle. Unlike higher eukaryotes, the cytoskeleton does not appear to disassemble during mitosis. The kinase complex, CDK/cyclinB, which controls progression through these cell cycle stages in eukaryotes, was identified in *K. brevis* by western blotting and by co-immunoprecipitation experiments. Unlike higher eukaryotes, which express cyclinB only late in the cell cycle, cyclinB was present in *K. brevis* at similar levels throughout cell cycle. This behavior was confirmed by immunocytochemistry, which demonstrated expression of cyclinB throughout the cell cycle and an unusual cytoplasmic localization during both interphase and mitosis. Unlike higher eukaryotes, the cytoplasmic pool of cyclinB was not translocated to the nucleus prior to mitosis. Together these results identify key components of the eukaryotic cell cycle in *K. brevis*, modified to accommodate dinomitosis and independent of transcriptional control of cyclinB. Elucidating the cellular mechanisms that control the process and rate of cell division is critical to understanding the growth phase of *K. brevis* blooms.

**** BREVETOXIN -2 AND -3 VARIABILITY FROM STRAIN OF KARENIA BREVIS ISOLATED FROM FLORIDA AND TEXAS USING TANDEM MASS SPECTROMETRY**…
Nicole Wiggins-Mitchell et al. (Peter Moeller, Mark Busman and Steve Morton)

ABSTRACT

Cultures of *Karenia brevis* isolated from Texas and Florida were analyzed by tandem mass spectrometry for the presence of brevetoxin -2 and -3. All cultures were grown under identical environmental conditions. Once each culture reached late-log growth phase, 30ml were removed and extracted for toxin determination. The method for the LC-MS/MS utilized reversed phase chromatography coupled to tandem mass spectrometry at atmospheric pressure ionization on a SCIEX API III triple quadrupole mass spectrometer. The chromatography was spread over a 35-minute gradient ranging from 50% to 95% aqueous methanol. The column used for optimum separation was a 125 x 4 Hewlett Packard C-18. The extracts were bracketed between standards of brevetoxin -2 and -3 ranging in concentration from 11.2mM to 0.00011mM. The limit of quantification was found to be 0.0011mM and the limit of detection was 0.0011mM. The following masses were monitored for the presence of the toxins: 807D, 860D, 878D, and a parent mass of 896.5D. Expected retention times were 28.5 minutes for brevetoxin-2 and 28.0 minutes for brevetoxin-3. From this analysis, it was determined that the total amount of brevetoxin in the cultures from Texas are significantly higher than those isolated from Florida. This data could prove important since historically the harmful algal blooms in Texas are much smaller in size than those from Florida, yet the Texas blooms typically result in larger fish kills.

**** ISOLATION, CHARACTERIZATION AND CURRENT CHEMICAL STRUCTURAL INFORMATION ON A WATER SOLUBLE TOXIN DERIVED FROM *Pfiesteria piscicida*** … Peter Moeller et al. (John Ramsdell, Steve Morton, Brad Mitchell and Steven Eaker)

ABSTRACT

The structure and function of marine biotoxins are inextricably linked. To measure, characterize or chemically modify the activity of a particular toxin, its molecular structure must be determined unambiguously. Bioassay guided extraction and fractionation schemes have yielded discrete water soluble toxic fractions from *Pfiesteria piscicida*. As a video clip will show, these chemical fractions exhibit strong icthyotoxic activity to Sheepshead minnow, clearly demonstrating a chemical toxicity associated with *Pfiesteria* extracts. However, isolation and purification of the toxic compound(s) from salt-water extracts is a complicated process. Small sample sizes, molecular degradation processes and a consistent loss of activity over the time period required for purification and structural elucidation have required the use of innovative chromatographic methods in our efforts to complete structural analysis as quickly as possible. We have been able, using rapid novel purification methods, to provide partial MS and NMR data on active fractions prior to molecular degradation. Archived NMR spectra obtained

from multiple cultures over time have demonstrated the presence of a consistent molecular system existing in the active fractions. Though obtained on microgram quantities from purified fractions, the data obtained to date has provided interesting structural information, providing clues to the identity of functional groups associated with the toxin(s). Identification of these functional groups in turn provides us opportunities to chemically stabilize the toxin(s) allowing the design of preparative scale isolation and purification schemes. We continue large scale culturing to provide enough material to finish the structural characterization using ^{13}C and ^{1}H NMR analysis.

**** ALGICIDAL ACTIVITY OF A WATER-SOLUBLE COMPOUND EXTRACTED FROM *Trichodesimium thiebauti*** … Steve Morton et al. (Steven Eaker, Michelle Hsia, Tracy Schock and Peter Moeller)

ABSTRACT

Trichodesmium is an enigmatic cyanobacterium genus of at least 5 taxa ubiquitous in the tropical/subtropical reaches of the ocean. There are reports of benthic mortality, fish avoidance, bactericidal action and a variety of toxin effects have associated with various *Trichodesmium* blooms. This research focus on a water-soluble compound extracted from field collected *Trichodesimium* populations that were tested against a number of different phytoplankton families. This fraction inhibited the growth of *Dunaliella tertiolecta, Rhodomonas salina, Thalasiosira weisflogii, Chaetoceros neogracile, Emiliania huxleyi, Karenia brevis,* and *Prorocentrum micans*. Culture of *T. thiebauti* displayed similar algicidal activity as field collected material. However, cultures of T. *erythraeum* displayed no algicidal activity. Since *T. thiebautii* is found primarily offshore, and *T. erythraeum* is a more nearshore species, the location and species composition of a *Trichodesmium* event may have profound implications for nutrient flow into other phytoplankton. Specifically, one species of *Trichodesmium* appears to permit *K. brevis* growth, *T. erythraeum*, whereas another species, *T. theibautii*, produces an algicidal compound.

**** THE USE OF VOLUNTEERS TO MONITOR PHYTOPLANKTON**… Katherine Schaefer and Steve Morton

ABSTRACT

Monitoring networks have been created to test specific environmental components on a large-scale basis. This will allow scientists to focus on specific problem areas for future study. Phytoplankton monitoring networks have become a growing trend for coastal states to monitor their waters. These networks allow scientists to create a species list for their state as well as learn of the potentially harmful algae that exist in their waters. The use of volunteer monitoring programs is not a new idea. Some coastal states, Maine and California, have found ways to make their monitoring programs remain successful based on their initial goals for as long as a decade in California. The South Carolina Phytoplankton Monitoring Network (SCPMN) commenced in January 2001 with three school groups monitoring waters in Charleston County. This program has expanded to nineteen schools and three citizen groups within its first year of existence**.** Monitoring programs are beneficial to both the scientific community as well as the general public.

**** ORAL EXPOSURE OF BREVETOXIN IN MICE: PROTECTIVE EFFECTS OF CHOLESTYRAMINE** … Gordon et al. (Yasmine Bottein, Ricky Woofter, Stacie Dover and John Ramsdell)

ABSTRACT

There is a need to develop prophylactic treatment to alleviate the toxicological effects of dietary exposure to marine algal toxins. Dietary treatment with cholystyramine (CHYS) is one possible means of either binding or disrupting enterohepatic circulation of toxins and thereby mitigate the toxic symptoms. While CHYS has been used successfully to block the toxic effects of mycotoxins and other toxicants, there is little supportive experimental evidence with algal toxins. In this study, the hypothermic response to brevetoxin and biomonitoring toxin levels in blood are used as endpoints to quantify toxicity. Female CD-1 mice were implanted with radiotransmitters (Data Sciences) to continuously monitor core body temperature (T_c). One week after recovery, mice were dosed with brevetoxin (PbTx-3) by oral gavage. Doses of 230 and 300 ug/kg PbTx-3 were nonlethal but caused substantial reductions in T_c of 2.6 and 3.3 °C, respectively within 20 min and full recovery within 60 min. The mice were terminated 4 hr after dosing and blood was taken by cardiac puncture and collected on blood cards for measurement of PbTx-3 by radioimmunoassay. PbTx-3 blood levels were 17 and 45 nM in mice dosed with 230 and 300 ug/kg PbTx-3, respectively. A first set of preliminary experiments determined that dosing of CHYS by oral gavage of mice (25g/kg in a volume of 0.4 ml) one hour prior to brevetoxin exposure was not an effective route of

administration. Present studies are evaluating administrating CHYS incorporated in the diet and monitoring the hypothermic response and blood levels of the toxin.

**** VARIATIONS IN REACTIVITY OF rRNA-TARGETED PROBES AND TOXICITY FOR *Pseudo-nitzschia multiseries* GROWN IN NITROGEN- AND SILICATE-LIMITED CONTINUOUS CULTURES** … Peter Miller et al. (Greg Doucette and Christine Powell)

ABSTRACT

An isolate of *Pseudo-nitzschia multiseries* isolated from Monterey Bay, California, was cultivated in nitrogen- and silicate-limited continuous cultures over a range of specific growth rates spanning 0.2 – 1.2/day. A ribosomal RNA-targeted probe (muD1) specific for *P. multiseries* was applied to these cultures using fluorescent whole cell (WC) hybridization and sandwich hybridization (SH) techniques. A receptor binding assay for domoic acid (DA) was also applied to cells and culture media. Results of the WC assay showed that fluorescence intensity of cells grown under N limitation exhibited a marked decrease with declining growth rate, whereas the fluorescence intensity of Si-limited cells did not vary appreciably over the range of growth rates examined. Results of the SH assay also revealed some reduction in signal for N-limited cultures, but not to the extent expected based on the weak fluorescence observed in the WC probe treatments for the slowest growing cultures. Interestingly, for the Si-limited cultures, results of the SH assay indicated an increase in reactivity as growth rate decreased. At the fastest growth rates, both N- and Si-limited cultures reacted similarly in the SH assay, with values approaching that of a typical nutrient replete batch culture. Results of the toxin assays generally showed that DA equivalents per cell increased as growth rate declined for both N- and Si-limited cultures, although maximum values obtained for the N-limited cells were 10- to 100-fold less than those for Si-limited cells. Changes in the amount of DA activity in the Si-limited culture media mirrored those of the cellular values, with more dissolved DA being present in slower growing cultures. In contrast, dissolved DA was not detected in the media of any N-limited culture. Implications of these findings are discussed with respect to analysis of natural samples.

**** NOAA's MARINE BIOTOXINS ANALYTICAL RESPONSE TEAM**… Tod Leighfield et al. (Fran VanDolah, Greg Doucette, Peter Moeller, Steve Morton, Nikki Wiggins and John Ramsdell)

ABSTRACT

NOAA's Marine Biotoxin Analytical Response Team at CCEHBR provides a national response capability to assess potentially toxic organisms and to verify exposure of living marine resources to algal toxins. Because algal blooms and their associated morbidity and mortality events are largely unpredictable and often novel, an adequate response requires rapid communication, a multi-disciplinary team of scientists responsible for designing and implementing an event-specific solution, and advanced toxin detection capabilities. The Analytical Response Team at CCEHBR provides support to federal and state governments as well as non-government organizations for marine toxin analyses in response to unexpected HAB-related events. Since 1993 the Analytical Response Team has provided analyses for 35 different investigations related to algal blooms and algal toxins, including their impacts on fish, marine mammals, birds, and human exposures. Close collaboration with NOAA's Working Group on Marine Mammal Unusual Mortality Events resulted in the successful identification of domoic acid as a causative agent in the sea lion mortality event in California (1998) and subsequent multi-species events in 2000 and 2002, and confirmed brevetoxin involvement in mortalities of bottlenose dolphins along the Florida panhandle (1999-2000) and of manatees on the west coast of Florida (1996, 2002). Analyses are performed free of charge on a collaborative basis to fulfill NOAA's national HAB response mission. For information or to request assistance, contact the Analytical Response Team coordinator, Tod Leighfield (tod.leighfield@noaa.gov) or visit our website at http://www.chbr.noaa.gov/CoastalResearch.html.

**** EVALUATION OF TOXICITY IN RAPHIDOPHYTES ISOLATED FROM THE UNITED STATES, CANADA, AND JAPAN**… Allen et al. (Steve Morton and Peter Moeller)

ABSTRACT

In Asia and Europe, raphidophytes have been associated with pen-reared fish mortality; more recently, raphidophytes were linked to an estuarine fish kill in Delaware, USA. Production of brevetoxins and reactive oxygen species has been attributed to certain raphidophyte strains, but the mechanism(s) by which raphidophytes kill fish is poorly understood. We evaluated the ichthyotoxic potential of ten clonal cultures of raphidophytes,

including <Heterosigma akashiwo>, <Chattonella subsalsa>, <C. marina>, and <Fibrocapsa japonica>, isolated from estuaries and brackish ponds of the eastern USA, western coastal waters of Canada, and coastal waters of Japan. Cultures were grown at 23?C in f/2-Si or L-1 medium (salinity 15-30; 100 µmol m^{-2} s^{-1} irradiance with a 12:12 light: dark cycle), and were evaluated for production of brevetoxins, superoxides, and hydrogen peroxide. HPLC/MS analysis and receptor binding assays yielded no detectable brevetoxins, and repeated acute toxicity microassays using sheepshead minnows (<Cyprinodon variegates>) showed no toxicity toward test fish. Low levels of superoxides and hydrogen peroxide were detected from some isolates; however, the concentrations were too low to cause fish stress or mortality. The data indicate that under the culture conditions evaluated, these isolates had low potential to cause physiological stress in fish. In ongoing studies we are evaluating the effects of various environmental conditions (e.g., nutrient availability, light regime) on the potential for production of brevetoxins and reactive oxygen species in these and other raphidophyte isolates.

**** COMPARISON OF RECEPTOR AND IMMUNOASSAYS FOR BREVETOXIN DETECTION IN THE BLOOD OF EXPOSED ANIMALS** … Ricky Woofter et al. (Yasmine Bottein, Peter Moeller and John Ramsdell)

ABSTRACT

A radioimmunoassay (RIA) has been developed using a sheep anti-brevetoxin to evaluate detection of brevetoxin from the blood of animals exposed to the toxin. The assay uses a 1/4000 dilution of sheep antiserum, 0.4 nM [3H]-PbTx-3, and goat antisheep IgG-cellulose with separation on glass fiber filters. The RIA has high affinity for PbTx-3 (EC50= 1.4 $\pm$ 0.2 nM, n=6) and recognizes both type 1 and type 2 brevetoxins, but not ciguatoxin. Comparison of RIA to receptor assay yields a rank order of PbTx 6 > 3 = 2 = 9 > 1. Thus, the two assays provide comparable values for the commonly occurring PbTx-2 and 3 as well as PbTx-9, while showing differences for PbTx-6 and PbTx-1. We next compared the two assays to measure brevetoxin in the blood of exposed mice. ICR female mice (20 g) were treated intraperitoneal with PbTx-3 or PbTx-2, symptoms recorded and blood collected 4 hours after exposure. Blood spots (0.1 ml) were extracted and brevetoxin measured by RIA and receptor assay. In mice treated with PbTx-3, receptor assay and RIA gave comparable blood brevetoxin levels (30 *vs* 42 nM; 180 ug/kg at 4h); however, in mice treated with PbTx-2, the RIA gave about 10-fold higher levels than measured by receptor assay. In PbTx-3 treated animals, blood brevetoxin values measured by both receptor assay and RIA correlated well with symptomatology. However, in PbTx-2 treated animals, brevetoxin levels measured by receptor assay but not RIA, correlated with symptomatology. The apparent overestimation of blood brevetoxin by RIA in PbTx-2 treated mice may be the result of a PbTx-2 metabolite; either a bioactive form with greater reactivity to the antibody or a potential biomarker that sustains high circulating levels. Taken together these results show the value of tier-based testing for brevetoxin: antibody methods provide a good screening method that may detect metabolites; receptor-based methods provide a good toxicity measurement and LC-MS/MS provides absolute confirmation of toxin congeners.

**** DEVELOPMENT AND APPLICATION OF LSU rRNA PROBES FOR *Karenia brevis* IN THE GULF OF MEXICO, USA** … Christina Mikulski et al. (Greg Doucette and Steve Morton)

ABSTRACT

A fragment of the large-subunit ribosomal RNA gene (LSU rDNA), consisting of 969 bases and spanning the D1-D3 variable regions, was sequenced from five cultured isolates of *Karenia brevis*. There were no sequence differences among any of the isolates, which originated from the Texas Gulf coast (Texas B5 and SP3), the Florida Gulf coast (PNS and NOAA1), and the East coast of Florida (JaxC5). A consensus sequence was then compiled and compared to a previously published sequence from *K. mikimotoi* (Genbank #AF200682), the closest known phylogenetic relative to *K. brevis* and a frequently co-occurring species, for the purpose of identifying unique *K. brevis* sequences. Fluorescently-labeled (FITC) oligonucleotide probes targeted to LSU rRNA and ranging in length from 18-22 bases were designed to recognize these sites in *K. brevis*. All probes had at least two base pair differences, as compared to *K. mikimotoi*. Among seven probes tested using whole cell hybridization, one uniquely identified all nine *K. brevis* strains in hand to the exclusion of all other species tested. A lack of cross reactivity was confirmed for this probe against a Gulf of Mexico *K. mikimotoi* strain isolated in 2001during a *K. brevis* bloom off Sarasota, FL., as well as against other *Gymnodinium* species, dinoflagellates, diatoms, and a raphidophyte. Preliminary analysis of probed cells by flow cytometry revealed that *K. brevis* could be distinguished from *K. mikimotoi*. This oligonucleotide probe uniquely targeting *K. brevis* will be used in conjunction with probes for

algicidal bacteria to investigate algal-bacterial interactions in the Gulf of Mexico using flow cytometry, and will also be valuable for distinguishing *K. brevis* from morphologically similar species in the same natural community.

**** STRESS RESPONSES IN *Karenia brevis*: IDENTIFICATION AND RESPONSE CHARACTERIZATION OF STRESS PROTEINS AND ANTIOXIDANT ENZYMES**… Jeanine Miller et al. (Fran VanDolah)

ABSTRACT

The cellular mechanisms by which Florida red tide dinoflagellate, *Karenia brevis*, adapts to the adverse conditions encountered in coastal waters are largely unknown. The induction of stress proteins (hsps) and antioxidant enzymes is a primary and highly conserved biochemical response to encountered environmental stressors, including changes in temperature, salinity, pH, light, and turbulence. These hsps and antioxidant enzymes are highly conserved in all phyla and have been the subject of many studies in diverse organisms, however little is known of either class of protein in dinoflagellates. The current study has identified representatives of 3 superfamilies of proteins in laboratory cultures of *K. brevis* by western blotting: 2 superfamilies of hsps, a chaperone protein and low molecular weight (LMW) hsps; and 1 superfamily of antioxidant enzymes, the superoxide dismutases (SODs). Hsp 60, an important cellular chaperone protein, was demonstrated to be both constitutively expressed and induced in response to heat or oxidative stress in *K. brevis*. A LMW hsp, localized to the mitochondria (mitoshsp), was identified following heat stress. Induction of a second LMW hsp, localized to the chloroplast (chlshsp), is observed in response to oxidative stress. Two SODs, Cu/Zn SOD and Mn SOD, exhibit induction following heat or oxidative stress. These results demonstrate that both stress proteins and antioxidant enzymes are involved in the adaptive mechanisms of *K. brevis* and, with further response characterization and screening of field samples, may be applicable as suitable indicators of bloom health.

**** POSSIBLE BIOACTIVE COMPOUNDS PRODUCED BY *Pfiesteria*-LIKE DINOFLAGELLATES ISOLATED FROM FLORIDA**… Steve Morton et al. (Christina Mikulski, Peter Moeller, Steven Eaker and John Ramsdell)

ABSTRACT

Cultures of different species of *Pfiesteria*-like organisms (PLOs) were grown in 100 L batches cultures and harvested at late-log growth phase. Production of biological active substances by each culture was examined from both resulting cell mass and spent culture medium. Both cell mass and spent culture medium were passed though a silica column and eluted with an elutropic solvent series. Totals of 5 samples were collected for both the cell mass extract and spent culture medium. Each of the 10 extracts was tested for the possibility of bioactivity using both live assays and cell based assays. Live bioassays included brine shrimp and sheepshead minnows while cell based assay included the cytotoxicity assay and the GH4C1 reporter gene assay. A brine shrimp active polar compound was isolated from all strains. Subsequent structural analysis of this fraction showed this activity was due to DEHP, a man-made phthalate ester. From strain Lucy, a polar fraction was observed to be active on the sheepshead minnow assay. Strains of two cryptoperidinosoid species displayed a non-polar fraction which was active on the cytotoxicity and GH4C1 reporter gene assay but were not toxic to fish. This data provides initial evidence of bioactive substances from cultures of PLOs. Whether these organisms produce a toxic substance is presently unknown and will require future pharmacological and chemical investigations.

**** THE PUTATIVE *Pfiesteria* TOXIN ACTIVATES IMMUNE CELLS OF HUMAN AND FISH ORIGIN** … John Ramsdell et al. (Yasmine Bottein, Stacie Dover, Peter Moeller and Steve Morton)

ABSTRACT

The health hazards attributed to *Pfiesteria piscicida* point to the need to characterize bioactive substances capable of causing adverse effects produced by this organism. The initial work to identify a *Pfiesteria* toxin relied upon cell-

based screens for activity. Of the cell types investigated, the GH4C1 rat pituitary cell was the most sensitive to the toxin and this cell has subsequently been used to direct purification, characterize potential receptors and ionic conductances. Pharmacologic analysis has indicated that the putative *Pfiesteria* toxin activates a P2X7 purinergic signaling pathway. The prevalence of P2X7 receptors on immune cells suggests that immune cells may be primary targets for *Pfiesteria* toxicity. Because of the concern of *Pfiesteria*'s adverse effects on humans and finfish, our experimental cell types have shifted to immune cells, specifically monocytes, of human and fish origin. Current investigations are using two human promonocytic leukemia cell lines (human THP-1 and MONOMAC-6) and a fish monocyte cell line (RTS11). To identify primary signaling effects of the toxin, we have developed a real-time analysis microplate assay using the high molecular weight fluorescent dye YO-PRO-1 (quinolinium, 4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]-1-[3-(triethylammonio)propyl]-,diiodide) which permeates cells following the formation of cytolytic pores associated with P2X7 receptor activation. We have determined that *Pfiesteria* toxin induces YO-PRO-1 accumulation in both human and fish monocytes and current work is characterizing the pharmacological action through use of selective P2X7 agonist and antagonists. In the THP-1 cells, the downstream effect of *Pfiesteria* toxin induces the release of the proinflammatory cytokine interleukin-1beta and parallel work will evaluate this response in the fish cell line. Overall these studies are directed to provide new information on the action of *Pfiesteria* toxin on organisms and cell types that may be most relevant to the *Pfiesteria*-associated morbidity and mortality events.

**** POSSIBLE INFLUENCE OF *Pseudo-nitzschia australis* POPULATION AND TOXIN DYNAMICS ON FOOD WEB IMPACTS IN MONTEREY BAY, CA, USA** … Greg Doucette et al. (Christine Powell)

ABSTRACT

Blooms of the domoic acid (DA) producing diatom, *Pseudo-nitzschia australis*, are well-documented in Monterey Bay, CA, USA and have been associated with a wide range of food web impacts in this area. Nonetheless, it appears that comparably high *P. australis* concentrations observed year-to-year do not always lead to harmful effects of a similar magnitude. In August 2000, a massive *P. australis* bloom occurred in Monterey Bay, with maximum cell concentrations >2 x 10^6 cells L^{-1}, yet no unusual mortality events accompanied this bloom, in contrast to the mass mortality of California sea lions in 1998 associated with ten-fold lower *P. australis* levels. Distributions of *P. australis* cells and DA, as well as physico-chemical characteristics of the water column, obtained during the 2000 bloom event may provide a partial explanation for the reduced food web impacts. To date, horizontal and vertical profiles of cells and DA have revealed highly patchy populations of *P. australis* cells ranging in toxicity over two orders of magnitude from <1 to *ca.* 140 pg DA eq. $cell^{-1}$, accompanied by dissolved DA concentrations varying from undetectable to 40 micrograms DA eq. L^{-1}. We are presently examining these wide disparities in cellular toxicity over time and space in the context of changing oceanographic variables and nutrient fields during the bloom. Preliminary indications are that as cell concentrations increase and nutrient levels decline over time, DA tends to occur increasingly in the dissolved fraction, resulting in abundant yet relatively low toxicity cells that may reduce the amount of toxin available for entry into the food web.

**** GROWTH REGULATION IN *Karenia brevis*: A GENOMIC APPROACH**… Fran VanDolah et al. (Michèle Barbier, Tod Leighfield, Jeral Tyler, Bennie Haynes and Jeanine Miller)

ABSTRACT

Karenia brevis is a toxin producing dinoflagellate associated with red tides in the Gulf of Mexico. As a component of the Florida ECHOAB program, our work investigates cellular level controls that regulate the rate of proliferation of *K. brevis* blooms. Circadian control of the cell cycle imposes a maximum division rate of 1/day. Cell cycle entry appears to be cued by dawn, with cells entering S-phase 8-10 h after the onset of light. Progression through the cell cycle is dependent on the activity of the eukaryotic cell cycle regulator, cyclin-dependent kinase (CDK). However, the activity of CDK, controlled by the transcription of its regulatory subunit in most eukaryotes, appears to be regulated in *K. brevis* post-transcriptionally. This is of interest since the expression of other circadian-controlled dinoflagellate genes is under translational or post-translational control. Indeed, mRNA differential display of *K. brevis* genes yielded few differentially expressed transcripts over two cell cycles (diel cycles). Thus we have taken a genomic approach to investigating growth regulation in *K. brevis.* A *K. brevis* cDNA library (lZapII) was developed and sequencing of ~2000 expressed sequences (ESTs) has been completed. Over 30% of sequences have high homology to known gene sequences deposited in GenBank, including cell cycle, signaling pathway, metabolism, photosynthesis and structural genes. Approximately 40% have modest homology, while the remainder have low or

no homology to known genes. Our current work focusing on transcriptional control of growth regulatory genes in *K. brevis* will be discussed. *K. brevis* sequences are accessible to the research community at www.marinegenomics.org and available for research collaborations.

**** OBSERVATION OF *Prorocentrum lima* IN SOUTH CAROLINA** …Katherine Schaefer and Steve Morton

ABSTRACT

Prorocentrum lima has been found in several small creeks in South Carolina through weekly monitoring efforts from March 2001 to January 2002. Every time *P. lima* has been found in these creeks, it was always found to be present in the water column. Samples were studied under the microscope at 20x to prove that *P. lima* was present in these waters.

**** RELEASE OF INTERLEUKIN-1 BETA FROM ACTIVATED MACROPHAGE CELLS: A POTENTIAL IMMUNOTOXIC EFFECT OF THE PUTATIVE *Pfiesteria* TOXIN** … Stacie Dover et al. (Ana Clara Melo, Yasmine Bottein, Steve Morton, Peter Moeller and John Ramsdell)

ABSTRACT

Current research is being conducted to determine the biological consequences of exposure of macrophages to the putative *Pfiesteria* toxin. A toxin has been identified and extracted from a culture of the dinoflagellate *Pfiesteria piscicida* that induces the c-fos luciferase reporter gene transfected on GH_4C_1 rat pituitary cells. Analysis of this induction demonstrated selectivity consistent with the P2X7 receptor pathway. The P2X7 receptor is highly abundant on macrophages. Therefore, THP-1 cells, a human monocytic cell line that differentiates into activated macrophages are being cultured to investigate the biological consequences of this toxin. Our experimental design stimulates THP monocytes with 10 µg/ml bacterial lippopolysaccharide and 250 units/uL human interferon-gamma for 48 hours, so that they differentiate into macrophages capable of producing IL-1beta. The THP macrophages are then treated with either 300 uM 2'- & 3'-O-(4-Benzoylbenzoyl) adenosine 5'-triphosphate (BzATP), a selective agonist of the P2X7 receptor, or the toxin. Culture medium is collected over 30 minutes and the release of IL-1beta from the cells is measured using a solid phase sandwich ELISA for IL-1beta. Exposure to BzATP results in a 75% average increase of IL-1beta above the control levels. *Pfiesteria* toxin results in released IL-1beta levels up to 41% above the control. Preliminary results indicate that the action of BzATP is blocked by adenosine 5'-triphosphate-2',3'-dialdehyde, an irreversible antagonist of P2X7 receptors. Further investigation is underway to more fully characterize the action of the toxin to induce release of inflammatory cytokines.

**** EVIDENCE FOR THE OCCURRENCE OF PSP TOXINS IN NORTH ATLANTIC RIGHT WHALES (*Eubalaena glacialis*) AND THEIR ZOOPLANKTON PREY IN THE BAY OF FUNDY, CANADA** … Greg Doucette et al. (Lisa Hollen and Ashley Anderson)

ABSTRACT

Intensive study of the western North Atlantic right whale (*Eubalaena glacialis*) population over the past 20 years has yielded evidence of reproductive dysfunction in this highly endangered cetacean species. Among the factors identified as potentially contributing to this phenomenon, exposure to marine algal toxins has received little consideration. We recently initiated a study to investigate the possible occurrence of paralytic shellfish poisoning (PSP) toxins in *E. glacialis* and in the zooplankton assemblage comprising the majority of its diet. Samples of *E. glacialis* fecal material from at least ten different animals obtained during Aug./Sept. 2001 from the Bay of Fundy, Canada, tested positive for PSP toxins by both receptor binding assay and by HPLC-FD analysis, with levels frequently exceeding 20 µg STX equiv. per gram of feces. Zooplankton samples collected during the same time period were also shown to contain PSP toxins by both methods. Confirmation of PSP toxins in whale and zooplankton samples by mass spectrometry is currently underway. Additional data revealed the presence of toxic *Alexandrium* cells immediately before and during the sampling period, while PSP toxin levels in shellfish from nearby Cheney Passage, New Brunswick, exceeded regulatory limits over the same time frame. These findings provide compelling evidence for the occurrence of PSP toxins in both *E. glacialis* and its zooplankton prey assemblage, suggesting that additional studies are warranted to examine the trophic transfer of these biotoxins and their possible effects on the reproductive success of this endangered species.

**** IDENTIFICATION OF *Prorocentrum lima* AND OKADAIC ACID IN THE BLACK SEA**… Tod Leighfield et al. (Steve Morton, Amy Longstreth, and John Ramsdell)

ABSTRACT

A joint US-Russian collaboration sponsored by the US Civilian Research and Development Foundation has identified a HAB species responsible for diarrheic shellfish poisoning on the Russian coast of the Black Sea. Phytoplankton and shellfish were collected throughout the entire Russian coastline of the Black Sea by a joint US-Russian team comprised of scientists from NOAA, the Shirshov Institute of Oceanology, and Moscow State University. The dinoflagellate *Prorocentrum lima* was identified growing in association with macroalgae, which dominates many parts of the subtidal zone. *P. lima* appeared to have a substrate preference for *Dictyota dichotoma* and *Padina pavonica*, both species non-endemic to the Black Sea, as compared to the dominant indigenous species *Cystoseira barbata* and *Cladophora albida*. *P. lima* cells were also identified in mussel stomachs by microscopic examination. Other potentially toxic phytoplankton species (*Dinophysis*, *Pseudonitzschia, Cochlodinium*) were also observed. Extracts prepared from field isolated cultures of *P. lima* show okadaic acid-like activity using a colorimetric phosphatase inhibition assay. Analysis of hepatopancreas from *Mytilus galloprovincalis* for okadaic acid-like activity yielded okadaic acid levels well below the Canadian and the European Union action level of 2?g per g of digestive gland. Specifically, low levels of phosphatase inhibitory activity were observed in two of five samples collected from Orlyonok, and in fourteen of twenty-six samples collected from the Utrish Center for Marine Biotechnology and Aquaculture. Although many potentially toxin producing dinoflagellates are known to be present in Russian coastal waters, there are no regulations regarding contamination in seafood and no statistics available on human poisonings.

**** *Prorocentrum lima* IN NEW ENGLAND COASTAL WATERS: POPULATION DYNAMICS AND TOXICITY**… Maranda et al. (Steven Eaker, Tod Leighfield and Steve Morton)

ABSTRACT

The epibenthic/epiphytic dinoflagellate *Prorocentrum lima* is widespread in New England coastal waters. The abundance and seasonality of this toxin producer are followed within the planktonic and epibiotic community, bimonthly at eight sites along the coast of New England, USA. In an effort to evaluate the potential for diarrhetic toxins to contaminate shellfish resources, the digestive glands of wild and cultured shellfish collected at four of the stations are analyzed for okadaic acid content. Two hypotheses are being tested: 1) *P. lima* from New England coastal waters is toxigenic with respect to production of okadaic acid and derivative active compounds, and 2) Shellfish grown in suspension culture become contaminated with DSP toxins at a faster rate and at a higher level than wild shellfish. The seasonal distribution and associated toxin patterns in mussels and oysters will be presented for the first of this planned two-year study. The ultimate goal of this project is to help evaluate the likelihood of DSP incidents, and the necessity and extent of future monitoring efforts to allay public health concerns.

**** DISTRIBUTION AND OBSERVATIONS OF HAB SPECIES IN N-E BLACK SEA**… Alexander Vershinin et al. (Tod Leighfield, Steve Morton, and John Ramsdell)

ABSTRACT

The primary goal of a joint US–Russian collaboration was to identify toxic algae in Black Sea, to assess the risks to the public, and to investigate key factors governing algal proliferation. *Dinophysis rotundata, D. caudata, D. acuminata, D. hastata, D. fortii, D. norvegica* were found on the Black Sea coast at numbers up to 2300 cell/L during the spring phytoplankton bloom. These are cell densities known to be able to cause Diarrheic Shellfish Poisoning. Okadaic acid activity was demonstrated in *Prorocentrum lima* laboratory cultures collected from macroalgae epiphytes growing at Utrish shellfish farm, Black Sea. A bloom of fish-killing microalgae *Cochlodinium polykrikoides* was observed in August 2001 at Utrish. Although no fish kills were observed, this species may become important if caged fish aquaculture develops in this region. All observed harmful dinoflagellate species were found to develop during the warm period and their densities followed a mixotrophic dinoflagellate community which follows the rise of the diatom community. Two species that may cause Amnesic Shellfish Poisoning were found in Black Sea, *Pseudonitzschia pseudodelicatissima* and *P. seriata*, corresponding results on shellfish toxicity

were negative, however they can not be considered conclusive since toxin production is known to vary depending on conditions. Monitoring those species in 2000-2002 lead to the conclusion that we should expect their highest numbers during the depressions of the whole phytoplankton assemblage, with peak concentrations in summer and winter. For the first time a species from the genus *Alexandrium* was found in the Black Sea, it had short population outburst near the Utrish shellfish farm after heavy rains in January. We may conclude that a variety of toxic algae represent a serious threat to public health and marine fauna on the Russian Black Sea coast.

PUBLISHED:

**** REVIEW AND ASSESSMENT OF *IN VITRO* DETECTION METHODS FOR ALGAL TOXINS…** Fran VanDolah and John Ramsdell (JAOAC International 84(5):1617-1626)

ABSTRACT

Not available at this time (see Fran)

**** MODIFICATION OF THE CELL BASED ASSAY FOR BREVETOXINS USING HUMAN CARDIAC VOLTAGE DEPENDENT SODIUM CHANNELS EXPRESSED IN HEK-293 CELLS**…Elizabeth Fairey et al. (Biosensors & Bioelectronics 16:579-586)

ABSTRACT

Assays using living cells provide an effective means to generate activity measurements of toxins, especially in situations where the toxins are part of a complex mixture or in an unfamiliar form due to natural or synthetic derivatives or bioactive metabolites. An important step in the refinement of cell based assays is to simplify the cellular reactions to generate the functional response of interest. Advances in engineering functional responses in cells provide a means to direct the response of given toxins. In this report, we describe the homogeneous high level expression of the initial target for brevetoxin, the voltage dependent sodium channel in human embryonic kidney cells (HEK-293). HEK cells stably transfected with a 6.208 kb cDNA of human heart voltage-dependent Na+ channel (hH1a) were examined as an alternative to mouse neuroblastoma cells (N2A). The HEK-hH1a cells showed a reduced dependence on cofactors, increased sensitivity to brevetoxin and a useful means to assure absolute selectivity to the sodium channel. We next assessed the assay in a reporter gene format. Expression of a panel of minimal response elements as well as the c-fos promoter failed to provide a response to brevetoxin, indicating that the HEK cells lack a necessary intermediate signaling component. The expression of voltage dependent sodium channels in HEK cells is anticipated to provide enhanced performance for cell-based detection of toxins for drug and natural product discovery, biomonitoring and environmental monitoring.

**** CURRENT PROGRESS IN ISOLATION AND CHARACTERIZATION OF TOXINS ISOLATED FROM *Pfiesteria piscicida*** … Peter D. R. Moeller et al. (Environmental Health Perspectives 109, suppl. 5:739-744)

ABSTRACT

The isolation and purification of a toxic substances derived from *Pfiesteria piscicida* extracts are described. Four distinct bioassay systems were utilized to monitor bio-activity of the *Pfiesteria piscicida* extracts . These included a high throughput cell cytotoxicity assay and a reporter gene assay as well as assays using brine shrimp and fish. Using these bioassays to guide fractionation, we have isolated two distinct active fractions from *Pfiesteria* culture medium and cell mass extracts based on their solubility characteristics. We have identified and characterized a bio-active lipophilic substance from *Pfiesteria* derived extracts, as di-(2-ethylhexyl) phthalate (DEHP), a commonly used plasticizer. The source of this typically man-made substance has been identified as originating from Instant Ocean, (Instant Ocean, Aquarium Systems, Mentor OH) a commercially available seawater salt mixture used to make up our mass culture growth medium. We have developed chromatographic methodology to isolate a bio-active polar metabolite from the extracts and presently report the characterization of the activity of this substance. The molecular structural analysis of the polar active component(s) using mass spectrometry and nuclear magnetic resonance spectroscopy is currently underway.

**** CLASSIFICATION, NOMENCLATURE, AND IDENTIFICATION OF *Pfiesteria* AND *Pfiesteria*-LIKE SPECIES**… Steidenger et al. (Steve Morton) (Environmental Health Perspectives 109, suppl 5:661-665)

ABSTRACT

-See Steve Morton.

**** HEALTH AND ECOLOGICAL IMPACTS OF HARMFUL ALGAL BLOOMS: RISK ASSESSMENT NEEDS** …Frances M Van Dolah et al. (Human and Ecological Risk Assessment 7: 1329-1345)

ABSTRACT

The symposium session, *Indicators for Effects and Predictions of Harmful Algal Blooms*, explored the current state of indicators used to assess the human health and ecological risks caused by harmful algal blooms, and highlighted future needs and impediments that must be overcome in order to provide a complete risk assessment of their impacts. Six recognized human poisoning syndromes resulting from algal toxins (paralytic, neurotoxic, amnesic, diarrhetic shellfish poisonings, ciguatera fish poisoning, and putative estuary associated syndrome) impact human health through consumption of contaminated seafood, direct contact with bloom water, or inhalation of aerosolized toxin. Thorough health risk assessment for the variety of algal toxins is hampered to varying degrees because either the toxin has not been identified or indicators for exposure and effects remain poorly defined. Predicting the occurrence and determining the impacts of harmful algal blooms in coastal ecosystems are the two major ecological risk assessment needs. In the former case, the *hazard* is the suite of conditions that trigger bloom initiation, magnify bloom intensity or support bloom longevity, whereas in the latter case, the *hazard* is the algal toxin. In both cases, indicators (of triggering mechanisms, exposure, and effects) are better defined for some HAB species and toxins than others, but are by no means complete.

**** CELL CYCLE REGULATION IN A DINOFLAGELLATE, *Amphidinium operculatum*: IDENTIFICATION OF THE DIEL ENTRAINING CUE AND A POSSIBLE ROLE FOR CYCLIC AMP**… Tod Leighfield and Frances M. Van Dolah (Journal of Experimental Marine Biology and Ecology 262:177-197)

ABSTRACT

This research describes the diel phasing of the cell cycle in the dinoflagellate, *Amphidinium operculatum* Clapar☞de & Lachmann, and investigates the mechanisms that serve to link the cell cycle to the diel cycle. Unlike many dinoflagellates, *A. operculatum* has a high division rate of approximately 1 division□day^{-1}, that yields a nearly synchronous population, making it useful for population studies. When grown on a 16:8 h light:dark cycle, S-phase begins 10 hours and mitosis 14-16 h after the onset of light, as determined by flow cytometry. Alterations in the timing of the dark/light and light/dark transitions showed that the cell cycle is entrained by the dark/light transition, with the light/dark cue being uninvolved. Cells in logarithmic phase growth also undergo diel changes in cell size, reaching a maximum size late in the light phase, concurrent with mitosis. Stationary phase cells or cells blocked in G1 of the cell cycle with a cell cycle inhibitor, olomoucine, showed no size changes or reduced size changes over the diel cycle, suggesting a coupling of cell size to the cell division cycle. In *Euglena*, cAMP dependent signaling appears to mediate diel phasing of the cell cycle. Therefore, the role of cAMP in cell cycle control in *A. operculatum* was investigated. Measurement of intracellular cAMP by radioimmunoassay revealed that cAMP concentrations varied on a diel basis, but increases observed appeared to correlate with cell size increases, and did not correlate with light cues at the dark/light or light/dark transition. However, when cells were treated with the cAMP phosphodiesterase inhibitor, IBMX, cell cycle progression was inhibited at both the G1/S and the G2/M phase transitions. This is in agreement with the role of cAMP in the cell cycle control in higher eukaryotes and is the first report of the involvement of cAMP dependent signaling in the dinoflagellate cell cycle.

OTHER PAPERS OF INTEREST:

The following are papers that either skipped the in-house review or (more likely) skipped my notice before they were in print. Please see the corresponding authors if these papers spark your interest.

** **ECOLOGICAL CHARACTERIZATION OF A WIDESPREAD RED TIDE IN SOUTH CAROLINA ESTUARIES: A NEWLY OBSERVED PHENOMENON…** Lewitus et al. (Steve Morton) (Harmful Algal Blooms, Proceedings of Tasmanian conference)

** **AMNESIC SHELLFISH POISONING IN THE KINGSCALLOP, *Pecten maximus*, FROM THE COAST OF SCOTLAND…** Campbell et al. (Mark Busman, Nikki Wiggins, Peter Moeller, Steve Morton) (Journal of Shellfish Research 20(1): 75-84)

** ***Pseudo-nitzschia pseudodelicatissima* – A CONFIRMED PRODUCER OF DEMOIC ACID FROM THE NORTHERN GULF OF MEXICO…** Pan et al. (Mark Busman, Peter Moeller, Christine Powell, Greg Doucette)
(Marine Ecology Progess Series 220:83-92)

** **ACCUMULATION OF DOMOIC ACID ACTIVITY IN COPEPODS…** Tester et al. (Youlian Pan, Christine Powell, Greg Doucette) ("Harmful Algal Blooms 2001", Proceedings of the 9th International Conference on Harmful Algal Blooms, IOC-UNESCO, Paris)

** **MICROFLUORIMETRIC ANALYSIS OF A PURINERGIC RECEPTOR ($P2X_7$) IN GH_4C_1 RAT PITUITARY CELLS: EFFECTS OF A BIOACTIVE SUBSTANCE PRODUCED BY *Pfiesteria piscicida*…** Ana Clara Melo et al. (John Ramsdell) (Environmental Health Perspectives 109, suppl 5:731-738)